VASCULAR FIBRINOID IN CUTANEOUS "ALLERGIC" ARTERIOLITIS*

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The term fibrinoid refers to a number of different tissue reactions with diverse pathogenesis. At present its nature is a focus of interest and many theories have been advanced for its explanation. The term was first used by Neumann in 1880 to describe substances resembling fibrin in their tinctorial behavior. Recently special attention has been given to changes in the collagen or the ground-substance as a probable origin of fibrinoid. There are still, however, considerable differences of opinion on the question of the nature of fibrinoid changes. The studies dealt with in the present article refer exclusively to vascular fibrinoid and were carried out on vascular lesions observed in a group of skin eruptions previously described by us under the term cutaneous allergic arteriolitis. The studies were mainly carried out on fixed tissues. In this way there were more opportunities of comparison with the data mentioned in the literature, since most information on fibrinoid has been obtained by studies on such material. It should be stressed that our findings are not necessarily applicable to fibrinoid changes described in many situations in other diseases.

Arteriolitis (Vasculitis) Allergica Cutis.

The vascular changes found in this group, largely confined to the skin, give rise to superficial cutaneous lesions. They occur in a number of skin eruptions of greatly different aspect, which, using the characteristic vascular alterations as a common denominator, can be classified into one and the same category (1, 2, 3). A number of more or less regularly recurring common cutaneous characteristics were noted in the various clinical types. As such were demonstrated: a) a tendency towards cutaneous hemorrhages; b) inflammatory changes of a transitory or more persistent character; c) the presence of an urticarial component. The eruptions were usually symmetrically distributed and often showed an episodic course. The lesions occurred mainly on the extremities, with occasional scattered elements on the torso. The group included skin eruptions ranging from anaphylactoid purpura, the symptoms described by Gougerot to cutaneous eruptions which have not been classified or have been classified erroneously (*e.g.*, some cases of Mucha's disease (4)). Differentiation into individual types of eruption based on an analysis of the clinical cutaneous changes meets with many difficulties. Personally we are inclined to regard both skin eruptions already known and belonging to this group and those newly found, as clinical variants (hemorrhagic type, papulo-necrotic type, polymorphous nodular type etc.) of one and the same cutaneous vascular condition. As far as the pathogenesis is concerned, most authors are of opinion that an allergic or related mechanism (microbes, drugs etc.) may be involved (5).

As pointed out previously, the histological substrate is dominated by rather characteristic vascular changes occurring predominantly in the small dermal vessels Fig. 1 (6, 7). The endothelial cells of all the affected blood vessels are markedly swollen. Inflammatory infiltrates of widely varying density, predominantly of polymorphonuclear leukocytes but also of cells resembling lymphocytes and a few eosinophils, occur in the vascular walls proper, but to a greater extent around the vessels. Nuclear disintegration of a considerable number of leukocytes is a prominent feature. In the majority of cases "fibrinoid" changes of the vascular walls, often reaching into the surrounding tissue are found. Generally speaking the vascular lesions have an exudative character and are everywhere of about the same duration.

Methods and materials

Depending on the staining method to be used the biopsy specimens were fixed in formol or alcohol; frozen sections (cryostate) were also used. The sections were 8 μ thick; staining methods were: hematoxylin eosin, the P.A.S. technic without and with treatment with diastase, Mallory's phosphotungstic acid-hematoxylin method (in Pearse's modification for formol-fixed material), Masson's trichrome stain and Gomori's silver impregnation technic. A number of sections were stained with toluidin blue (frozen sections), Weigert's fibrin method (alcohol fixation), Heidenhain's iron hematoxylin, the P.A.S. reaction after acetylation, the coupled tetrazonium reaction (alcohol fixed material), without or after heating and benzoylation, and the Feulgen technic. A number of sections fixed in alcohol were subjected to trypsin extraction after Fawns and Landells (8).

The histological investigations were performed on biopsy material from 15 cases of cutaneous allergic arteriolitis. It appeared that the method of fixing used for the various staining technics sometimes rendered mutual comparison difficult. Care was taken to compare as far as possible ma-

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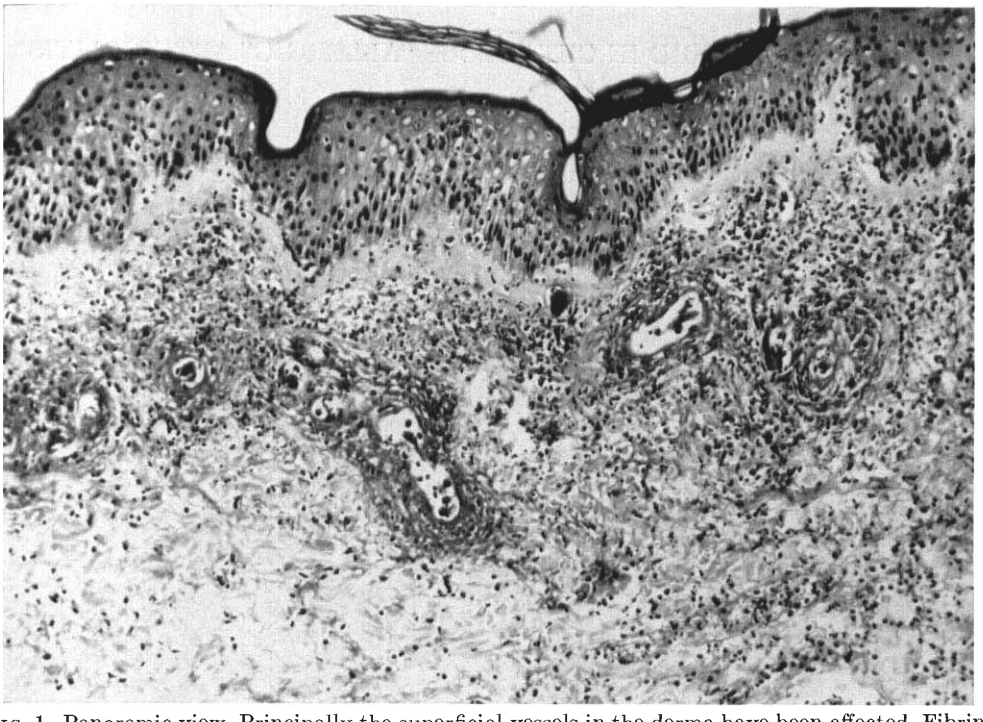

FIG. 1. Panoramic view. Principally the superficial vessels in the derma have been affected. Fibrinoid changes in the vascular walls and inflammatory infiltrates with marked nuclear disintegration. Hematoxylin and eosin. (× 120).

terials fixed by the same method. As a rule more than one biopsy specimen was taken from the same patient.

The perivascular connective tissue

In Gomori's silver impregnation a marked splitting up of perivascular collagen fibers was found. The newly formed fibers were argentophilic and will be described as "pathological" collagen. They, in turn, split further, leading to the formation of numerous new units that blended into the perivascular reticulin consisting of branching reticulin fibers (fig. 2). Thus an extensive argentophilic meshwork had developed around the vascular lumen, showing a reticular pattern that became especially distinct if the vessel had been tangentially cut. The "pathological" collagen showed argentophilic fibers that were considerably thicker than the reticulin fibers. After elimination of the protein component by digestion with trypsin the "pathological" collagen showed a P.A.S.-positive reaction, as occurs with reticulin.

The protein component

The fibrinoid material in question stained red with eosin, reddish-brown with the coupled tetrazonium reaction after heating (80°) and benzoylation and blue with Mallory's phosphotungstic acid-hematoxylin (see also fig. 3, 4 and 5). From this can be concluded (Pearse (9)) that at least the major part of the protein component consisted almost certainly of fibrin. These findings are comparable to those of Gitlin and Craig, obtained by means of Coon's and Kaplan's fluorescent antibody method, in fibrinoid of various origins (periarteritis nodosa, visceral lupus erythematosus and rheumatic nodules) (10). By means of this interesting technic, which however shows few if any cytological and histological details, these authors demonstrated that fibrin formed a considerable part of the fibrinoid material found in these diseases.

After it had been found that at least the major part of the protein component of the fibrinoid changes in arteriolitis allergica cutis consisted of fibrin, a number of other histological technics were applied to the fibrinoid material. Feulgen's reaction was occasionally feebly positive, possibly due to the marked nuclear disintegration of the leukocytic infiltrates. In frozen sections stained with toluidine blue (pH 5) affected vessels of somewhat larger caliber situated in the lower part

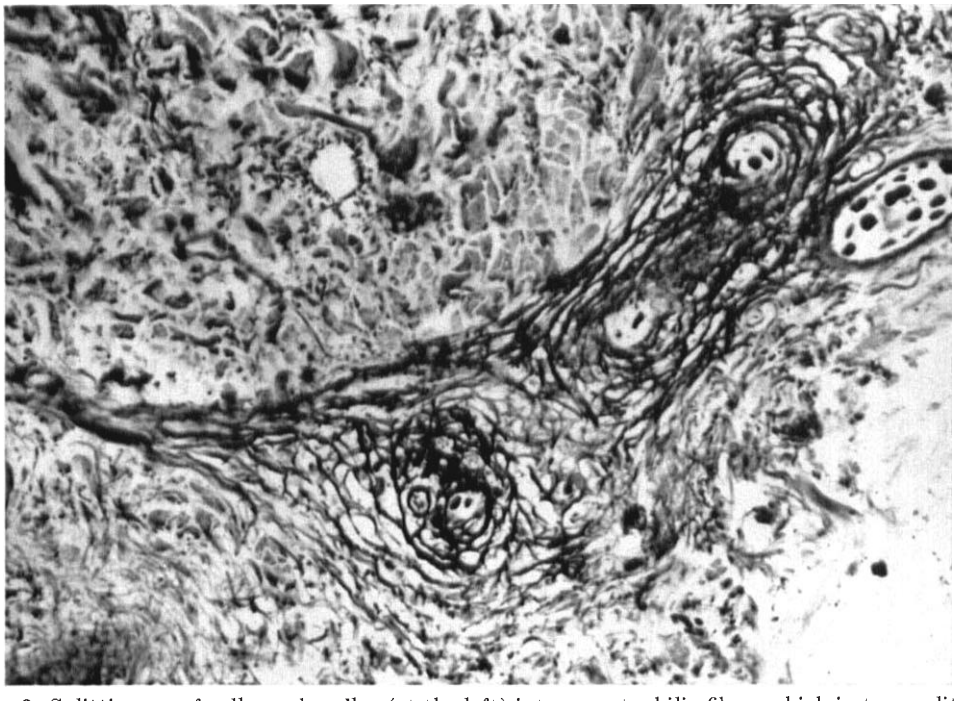

FIG. 2. Splitting-up of collagen bundles (at the left) into argentophilic fibers which in turn split and lead to the development of numerous new units forming an argentophilic perivascular meshwork. Gomori's method. (× 300).

of the dermis showed some metachromasia. However these vessels were in a reparative stage and were frequently surrounded by a number of mast cells. Affected vessels of smaller caliber as seen in the stratum papillare and subpapillare, however, did not stain metachromatically in this way. The periodic acid leucofuchsin reaction of the "fibrinoid" substances after treatment with diastase —the leukocytic infiltrates appeared to contain large amounts of glycogen—was positive in both formol-fixed material (faintly pink) and frozen sections (reddish). At the same time alcohol-fixed sections were incubated at 37° C in a 0.5% crystalline trypsin solution in a phosphate buffer of pH 8. In control sections incubated in buffer without enzyme the protein component could be easily demonstrated. After incubating the sections for 10–15 minutes with trypsin, however, it appeared to be completely removed and P.A.S.-positive substances were no longer demonstrable in the vascular wall. Only the "pathological" collagen-reticulin meshwork was stained purplish-red.

The above findings indicate that the P.A.S.-positive substances were associated with fibrin. Studies on a series of artificial clots produced from fibrinogen in different ways led Gitlin and Craig (11) to suggest that included substances may be responsible for the P.A.S.-positivity of fibrin. The fact that in our cases the protein component of the fibrinoid appeared to consist entirely, or for the major part, of fibrin suggests that the P.A.S.-positive substances, the topographical distribution of which corresponded to that of the fibrin, may be partially derived from external sources (plasma-proteins?). After treatment of the sections with diastase the P.A.S. reaction of the fibrin remained positive (pink). The reaction could be completely blocked by acetylation. These findings suggest the presence of carbohydrate protein complexes.

Morphogenesis of the fibrinoid changes

By comparing the histological pictures obtained by a number of staining technics mainly used for demonstrating of fibrin and including the methods already mentioned, we have made an attempt to gain a better insight into the significance of the protein component in the morphogenesis of the fibrinoid in the vascular changes.

In hematoxylin-eosin preparations (fig. 3) the fibrinoid changes of the vascular wall and the

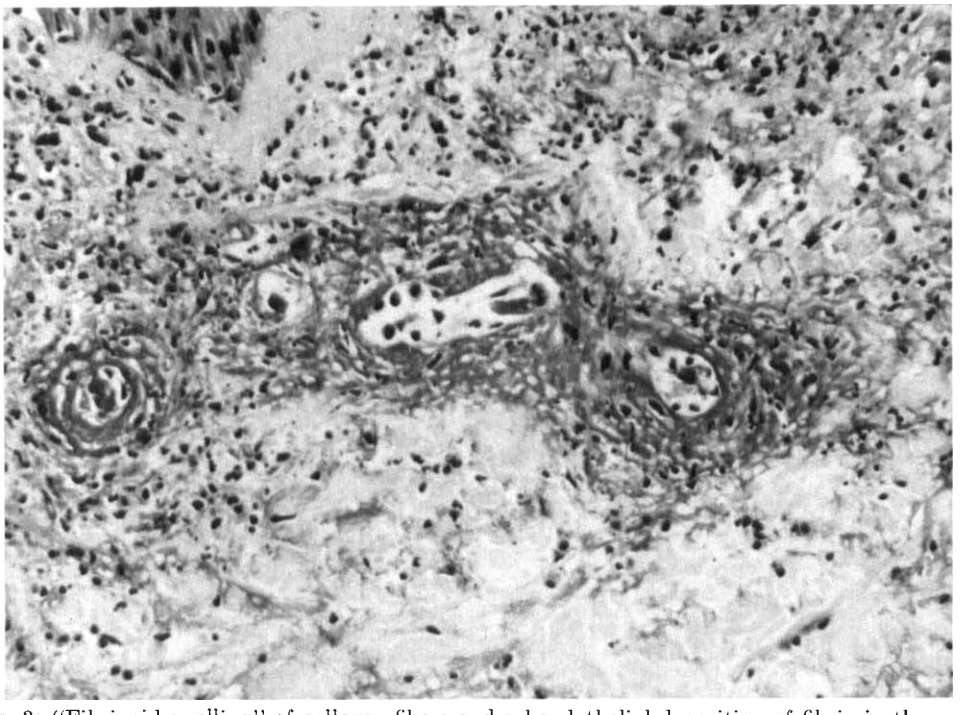

FIG. 3. "Fibrinoid swelling" of collagen fibers and subendothelial deposition of fibrin in the wall of an affected vessel. Inflammatory infiltrates with marked nuclear disintegration. Hematoxylin and eosin. (× 240).

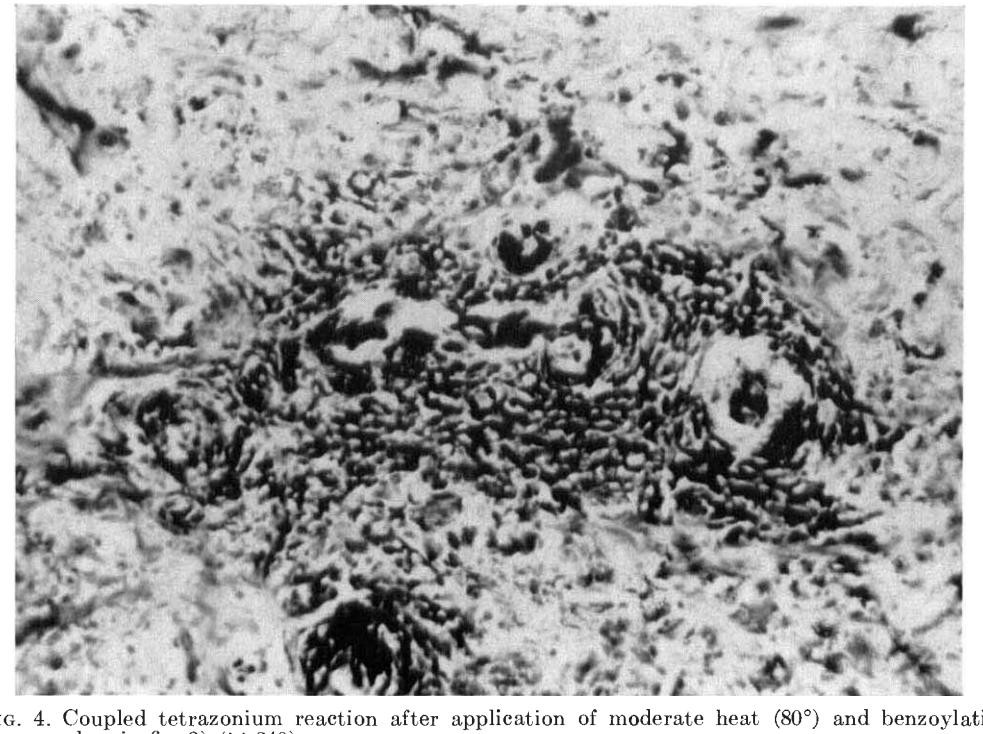

FIG. 4. Coupled tetrazonium reaction after application of moderate heat (80°) and benzoylation (same vessel as in fig. 3) (× 240).

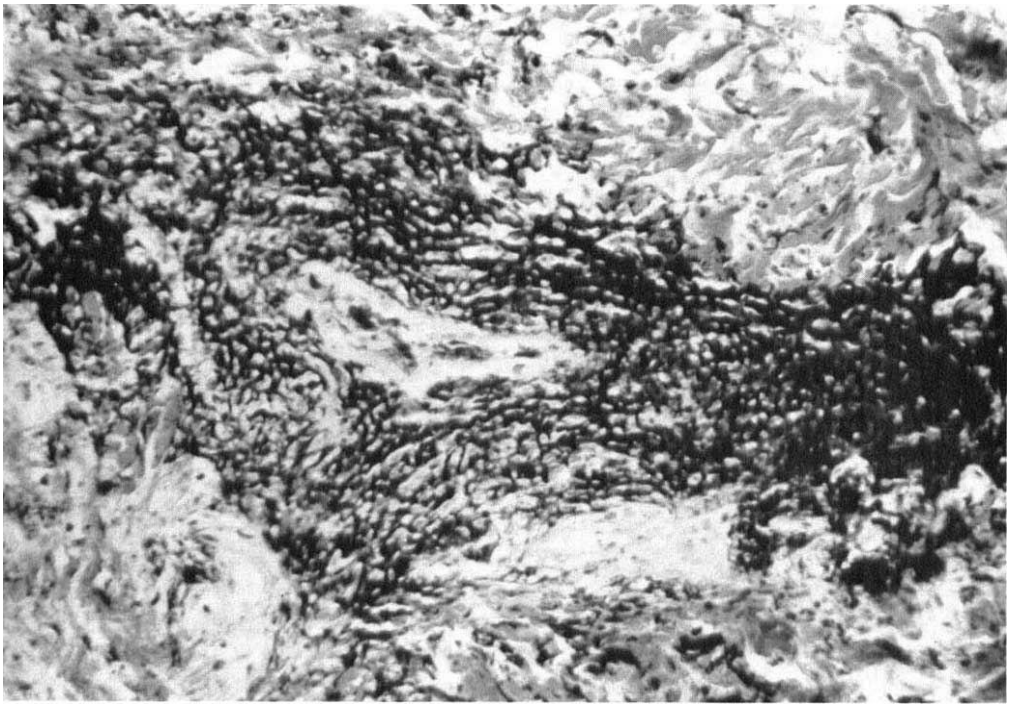

FIG. 5. Vessel cut lengthwise. Distinct reticular fibrin pattern. Mallory's phosphotungstic acid-hematoxylin. (× 240).

neighboring tissue appeared partly as eosinophilic homogeneously swollen collagen fibers which frequently showed a reticular pattern. In addition local deposits of eosinophilic substances occurred between the swollen fibers. Sometimes these deposits did not appear to be evenly stained red and showed more intensely stained areas. Occasionally in such cases also subendothelial deposition of eosinophilic material was seen (see also fig. 7). This "fibrinoid" stained more intensely with eosin than the rest, was slightly more refractile and showed a marked similarity to the material of "hyaline" thrombi. The latter were not infrequently observed in such sections within the small vessels. The staining methods used in the analysis of the protein component of the fibrinoid changes in and around the vascular walls revealed that the "hyaline" thrombi also were composed mainly of fibrin.

Slightly different pictures were observed with Mallory's phosphotungstic acid-hematoxylin stain, the coupled tetrazonium reaction after heating and benzoylation and Masson's trichrome stain. It appeared that these methods revealed a number of details which escaped attention in sections stained by other fibrin stains and hematoxylin-eosin. With Mallory's method the fibrinoid changes were frequently seen in the form of a dark-blue meshwork around the vascular lumen, showing a distinct reticular structure if the vessels had been cut lengthwise (fig. 5). On closer inspection it appeared that this fibrin pattern had developed due to the fact that a thin layer of the protein component (fibrin) had become attached to the fibers contained in the "pathological" collagen-reticulin meshwork. Sometimes the blue color of such a fiber was seen to disappear suddenly, while further along the fiber proper reappeared. Occasionally a thin coat of the blue-stained material could be observed on both sides of the original fibers when the latter were cut tangentially (fig. 6). Mallory's stain also demonstrated clearly that the fibrin may also be deposited in the form of "lumps" and/or band-like subendothelial depositions of "solid" material frequently in the presence of "hyaline" thrombi. This fibrin was also stained intensely blue. The fibrin "lumps" and the thrombi appeared not to be homogeneous, but to contain minute light-blue globules (plasma proteins?). The two forms of fibrin were usually found side by side, the reticular pattern being generally pre-

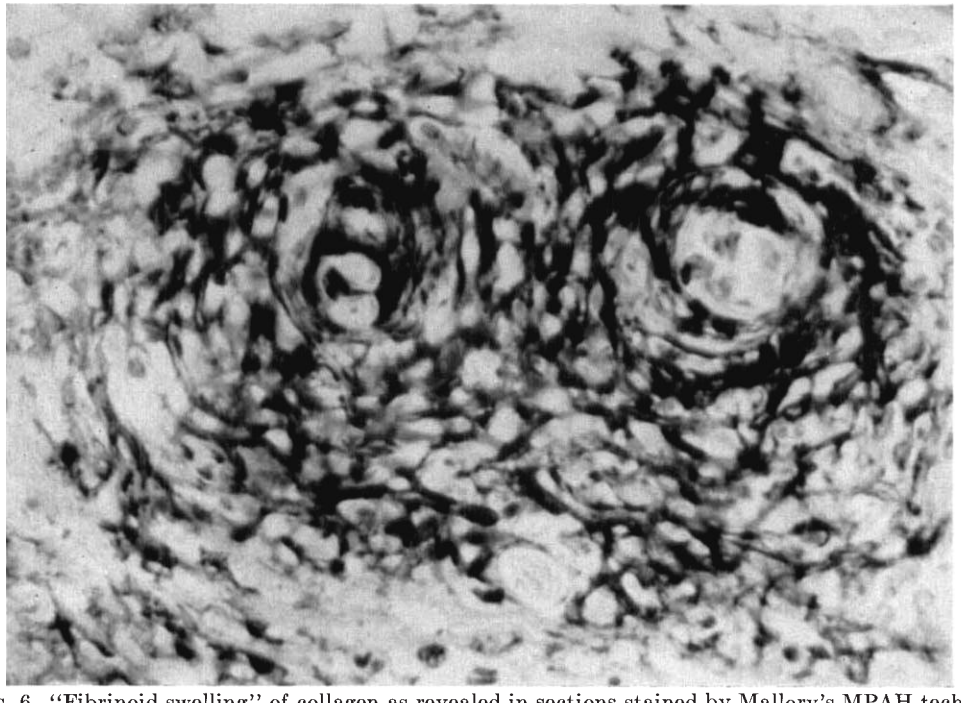

FIG. 6. "Fibrinoid swelling" of collagen as revealed in sections stained by Mallory's MPAH technic. Fiber; belonging to the perivascular collagen-reticulin meshwork showing incomplete coating with fibrin. (× 750).

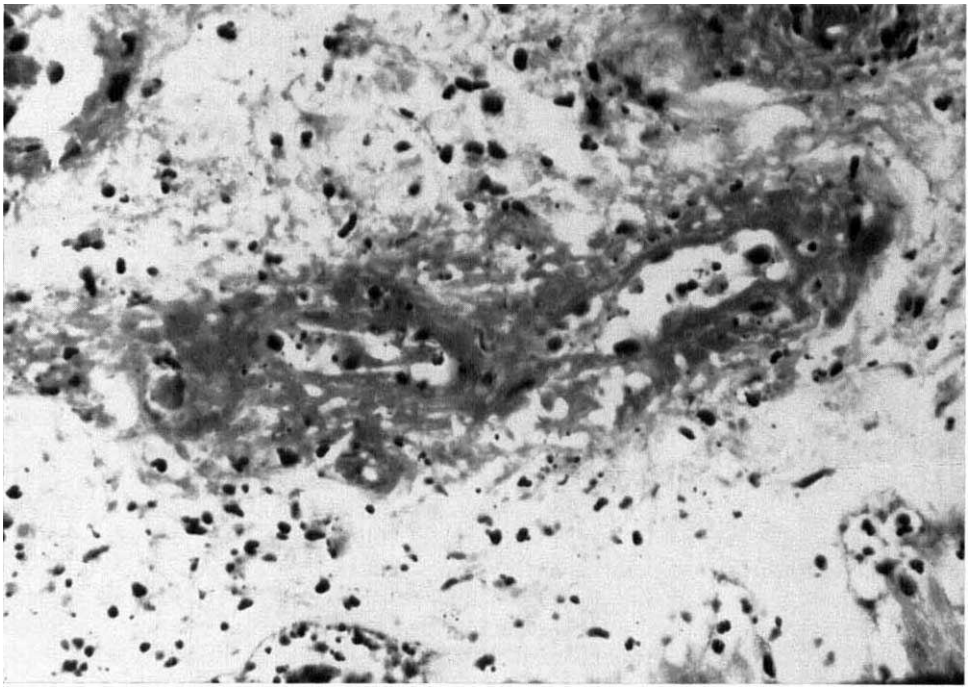

FIG. 7. Affected vessel giving the impression of fibrinoid necrosis. The largely structureless vascular wall contains "solid" fibrin as well as nuclear debris. Hematoxylin and eosin. (× 375).

dominant. Only rarely was fibrin seen in the form of crystalline needles scattered at random in the vascular wall and especially outside them. The findings obtained with the coupled tetrazonium reaction after benzoylation and moderate heating (fig. 4), corresponded to the pictures observed with Mallory's phosphotungstic acid-hematoxylin technic. Similar pictures were also found in the sections stained with Masson's trichrome stain, which is probably less selective than Mallory's method but yields marked contrasts.

From the above it appears that in vascular fibrinoid two forms of fibrin with different physico-chemical properties may be involved, *i.e.*, a more or less amorphous form with a tendency to attach itself to the fibers of the perivascular meshwork and an usually less pronounced "solid" form, which is slightly different from the first type and resembles the fibrin in the so-called "hyaline" thrombi.

DISCUSSION

Before discussing the rôle of fibrin in the morphogenesis of vascular fibrinoid a few remarks on fibrin and its staining properties in histological sections are indicated.

It appeared that there were a number of discrepancies between the histological pictures obtained with Mallory's technic, the coupled tetrazonium reaction after heating and benzoylation and Masson's trichrome stain on the one hand and some other methods used for demonstration of fibrin on the other. Whereas the staining of the fibrin attached to the fibers contained in the collagen-reticulin meshwork was discontinuous with the aforementioned technics, these fibers were stained uniformly when Weigert's fibrin stain or Heidenhain's iron-hematoxylin method were used. The discrepancies between both groups of fibrin stains, however, could be largely explained by the poor contrast especially in respect of collagen, yielded by the latter. This was also true for the sections stained with hematoxylin-eosin. At the same time it is worthy to note that sections stained by the coupled tetrazonium reaction applied without previous application of moderate heat and benzoylation—a technic used as a general reaction for proteins—showed pictures usually quite comparable to those obtained by the three fibrin stains particularly used in this study. This might indicate that the occurrence of atypical or non-stainable fibrins (Gitlin and Craig) has probably been only of minor importance in our investigations.

Thus, we believe that the methods we used for demonstrating fibrin give a fairly accurate picture of its occurrence in the histological sections. Based on the findings described in the present paper it seems possible to draw up a reconstruction along general lines of the morphogenesis of vascular fibrinoid in cutaneous allergic arteriolitis.

It appears that the so called fibrinoid swelling of connective tissue in these cases is largely due to a coating (or impregnation?) of pre-existent fibers belonging to a perivascular (argentophilic) meshwork, resulting from a splitting up of adjacent collagen fibers with fibrin. As pointed out these histological details do not become prominent when stains such as eosin are used which yield poor contrast in respect to collagen. In some hematoxylin-eosin preparations the wall of the affected small vessels showed hardly any structure and contained locally solid eosinophilic material (fig. 7). An analysis using various staining methods showed that this eosinophilic material consisted mainly of fibrin as well. This fibrin resembled the fibrin of "hyaline" thrombi. Such vessels showed pictures that resembled fibrinoid necrosis by virtue of the simultaneous occurrence of nuclear debris. The silver impregnation technic however did not reveal true necrosis of the "pathological" collagen and reticulin. The nuclear debris largely appeared to originate from disintegrated inflammatory cells. Moreover, on closer examination the muscular cells of the arterioles were usually found to be only partly impaired. These findings probably explain why in the conditions discussed a regeneration of the affected vessels, even if they appear to be severely damaged in hematoxylin-eosin preparations, is usually possible.

In the fibrinoid changes observed, destruction of connective tissue as seen in other types of fibrinoid apparently does not occur. The collagen alterations in arteriolitis (vasculitis) allergica cutis rather appear to be restricted to changes of a physico-chemical nature manifesting themselves in the form of a perivascularly situated argentophilic meshwork. It is largely in this meshwork that the deposition of fibrin as "fibrinoid" takes place. In a future communication the mechanisms involved in this process will be discussed. We are inclined to believe that the fibrinoid changes found in arteriolitis (vasculitis) allergica cutis may represent the "minimum" lesion as far as vascular fibrinoid is concerned.

SUMMARY

The vascular fibrinoid in arteriolitis (vasculitis) allergica cutis was studied. Skin biopsy specimens were fixed and paraffin sections treated with various histological and histochemical staining

methods. Two basic changes could be established: (1) "splitting up" of perivascular collagenous fibers into fine argentophilic fibrils ("pathological" collagen) resulting in the formation of a loose argentophilic perivascular meshwork; (2) deposition of fibrinoid material in and around the vascular wall. Histochemical staining reactions showed this material to consist largely of fibrin. It occurred in close association with the "pathological" collagen and/or as lumps and bandlike subendothelial deposits of "solid" fibrin.

The fibrinoid swelling of collagen as seen in hematoxylin-eosin stained sections was shown to be due to coating of "pathological" collagenous fibrils with fibrin. The deposition of solid fibrin in the vascular wall, together with nuclear debris from inflammatory cells, resulted in pictures simulating fibrinoid necrosis. However, no destruction of collagen could be demonstrated, the collagen changes apparently being restricted to alterations of a physico-chemical nature. It is suggested that the fibrinoid changes found in arteriolitis (vasculitis) allergica cutis represent the "minimum" lesion as far as vascular fibrinoid is concerned. Some further results of the histochemical studies are reported and a number of stains for fibrin is tested and mutually compared.

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